

Newly discovered paracrine functions of cathepsin D in cancer.

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Abstract

The lysosomal aspartic protease cathepsin D (cath-D) over-expressed and hyper-secreted by epithelial breast cancer cells is an independent marker of poor prognosis in breast cancer. We previously showed that over-expressed cath-D stimulates tumor growth and metastasis, and plays different essential roles in the multiple steps of tumor progression. Indeed, we demonstrated that cath-D can stimulate cancer cell proliferation, fibroblast outgrowth and angiogenesis, and can inhibit tumor apoptosis. Moreover our studies also indicated that a mutated cath-D devoid of catalytic activity remained mitogenic for cancer, endothelial and fibroblastic cells, suggesting an extra-cellular mode of action of cath-D involving a triggering, either directly or indirectly, of an as yet unidentified cell surface receptor. In this review, we detailed some literature concerning the role of stromal cells in cancer as well as our recent findings on the role of cath-D in fibroblast invasive growth highlighting its potential participation in the stromal reaction.

Introduction

Breast cancer is the leading cause of cancer death in young women. Hormono-therapy for hormono-dependent cancers and chemotherapy for hormono-independent or metastatic cancers are the most widely employed therapies. Hormono-therapy is relatively well tolerated, although problems of resistance developing often occur. Whilst chemotherapy can cure cancers detected early it is, however, less efficient when the tumor has metastasized and can be associated with marked toxic side effects. There is an urgent need to identify molecular targets to aid both in early diagnosis and in more effective treatment of this devastating disease. Tumor progression is the result of many consecutive and coordinated cellular functions. Tumor formation results from a gain of uncontrolled cellular proliferation associated with a loss of differentiation. In the process of metastasis, malignant cells have to invade the surrounding tissues, to disseminate into the blood or lymphatic circulation, to survive the immune defences and finally to grow in host tissues and induce angiogenesis. Cancer is a tissue-based disease in which malignant cells interact dynamically with multiple normal cell types, such as fibroblasts, macrophages, lymphocytes, adipocytes and endothelial cells. Tumor progression has recently been recognized as the product of an evolving cross-talk between tumor cells and its surrounding supportive tissue, the tumor stroma.

Different families of proteases have been largely implicated in invasion, extravasation, proliferation, angiogenesis, apoptosis and metastasis: the serine proteases such as uPA, uPAR and PAI1, the metalloproteinase family, the cysteine proteases such as cath-B and cath-L and the aspartyl protease cath-D.

Cathepsin D (cath-D) is a ubiquitous lysosomal aspartyl endoproteinase and its over-expression and hyper-secretion by human breast cancer cells has been extensively reported (Rochefort et al., 1987).

Many clinical studies indicated that cath-D is a prognostic factor predictive for metastasis risk in primary breast cancers (Rochefort, 1992; Ferrandina et al., 1997; Foekens

et al., 1999; Westley and May, 1999). Cath-D had been unequivocally demonstrated to be an independent marker of poor prognosis by quantification in the cytosol of breast cancer (Westley and May, 1999). However, far more conflicting data were found by immunochemistry, which presents the major interest as compared to cytosolic assay to define the cells which produce, or accumulate the antigens. Clearly, the major cath-D producing cells appear to be the cancer cell and stromal macrophages (Maudelonde et al., 1992). Cath-D production by fibroblasts is variable according to the publications. Some studies indicated that cath-D production is low compared to cancer cells as shown by immunochemistry (Maudelonde et al., 1992) and *in situ* hybridization with antisense RNA (Escot et al., 1996). By contrast, other studies indicated the prognostic role of cath-D over-expression by reactive stromal cells (Joensuu et al., 1995; Nadji et al., 1996; O'Donoghue et al., 1995; Têtu et al., 1999).

During its transport to lysosomes, the 52 kDa human inactive pro-cath-D is proteolytically processed into a single-chain intermediate active enzyme of 48 kDa located in the endosomes. Further proteolytic processing yields the mature active lysosomal protease which is composed of heavy (34 kDa) and light (14 kDa) chains (Richo and Conner, 1991). The human cath-D catalytic site includes two critical aspartyl residues (amino acid 33 and 231) located on the 14 kDa and 34 kDa chains, respectively (Metcalf and Fusek, 1993). Mannose-6-phosphate (Man-6-P) receptors are involved in cath-D lysosomal routing and in the cellular uptake of the secreted pro-cath-D (Von Figura and Hasilik, 1986), although cath-D may also be targeted to the lysosomes and endocytosed independently of Man-6-P receptors (Capony et al., 1994, Laurent-Matha et al., 1998). Pro-cath-D over-expressed by cancer cells is also secreted in excess and can be endocytosed by both cancer cells and by fibroblasts *via* M6P-receptors and other unidentified receptor(s) (Laurent-Matha et al., 1998).

During the last few years, we have shown, using an RNA antisense strategy that cath-D is a rate limiting factor for outgrowth, tumorigenicity and lung colonization of MDA-MB-231 breast cancer cells using an RNA antisense strategy (Glondou et al., 2002). Several reports have indicated that cath-D stimulates cancer cell proliferation (Vignon et al., 1986; Vetvicka et al., 1994; Liaudet et al., 1995) and increases metastatic potential *in vivo* (Garcia et al., 1990; Liaudet et al., 1994). Moreover we have reported that ^{D231N}cath-D mutated in its catalytic site, and hence devoid of proteolytic activity, is still mitogenic for cancer cells both *in vitro* in three-dimensional (3D) matrices and *in vivo* in athymic nude mice (Rochefort and Liaudet-Coopman, 1999; Glondou et al., 2001). Our immunohistochemical studies have indicated that cath-D, independently of its proteolytic activity, stimulates not only cancer cell proliferation by an autocrine and/or intracrine mechanism, but also tumor angiogenesis by a paracrine mechanism (Berchem et al., 2002). This highlighted, for the first time, the potential paracrine action of cath-D in the context of a tumor.

Stromal cells play a determinant role in cancer pathogenesis.

Tumor progression requires a continually evolving network of interactions between neoplastic cells and their microenvironment consisting of an extracellular matrix, a stroma composed of fibroblasts, adipose, vasculature and resident immune cells, and a mixture of cytokines and growth factors (Pupa et al., 2002). Current observations increasingly point to the contribution of stromal components to oncogenic signals that mediate both phenotypic and genomic changes in epithelial cells (Tlsty and Hein, 2001). Carcinoma formation from its earliest stages seems to depend on the ability of transformed epithelial cells to first recruit and then subvert a variety of stroma cells originating from adjacent normal tissue to become infiltrating or invading (Elenbaas and Weinberg, 2001). In many common carcinomas including breast, colon, stomach, and pancreas, stroma comprises the majority of tumor mass, in some cases accounting for over 90%. In various experimental tumor models, the microenvironment affects efficiency of tumor formation, rate of tumor growth, extent of invasiveness, and ability of tumor cells to metastasize. In breast carcinomas, the influences of microenvironment are mediated, in large part, by paracrine signalling between epithelial tumor cells and neighboring stromal fibroblasts (Elenbaas and Weinberg, 2001). In addition to receiving signals from epithelial cells, the stromal fibroblasts stimulate tumorigenesis by

releasing factors that act on epithelial tumor cells. The identification of genes that are selectively expressed in the stroma of malignant lesions has provided new insight into the molecular basis of stromal-epithelial interactions. Stromally expressed genes include growth factors, proteases and extracellular matrix proteins, all biological activities with potential roles in malignant progression (Liotta and Kohn, 2001). During the transition from normal tissue to *in situ* and invasive carcinoma, the micro-environment of the local host stroma is an active player. Stroma and tumor cells can interchange growth factors and also proteases, for example, matrix metalloproteases (MMPs) and urokinase plasminogen activator (uPA), to activate the adjacent extracellular matrix and, in turn, induce the selection and expansion of neoplastic cells (Liotta and Kohn, 2001). Increased stromal growth accounts for most of the increase in breast volume in the post-puberbal years and also occurs in breast cancer. The fibroblast is a major cell type of the stromal compartment and, as such, is intimately involved in orchestrating the stromal part of the dialogue in tissue homeostasis (Grinnell, 1994). The modification of fibroblasts in the stroma immediately adjacent to transformed epithelial cells has been documented in several tumor systems (Basset et al., 1990; Olumi et al., 1999; Shekhar et al., 2001).

Cath-D promotes fibroblast outgrowth in 3D matrices.

We recently demonstrated a requirement of cath-D for fibroblast invasive growth using a 3D co-culture assay with breast cancer cells either secreting or not secreting pro-cath-D (Laurent-Matha et al., 2005). Ectopic expression of cath-D in cath-D-deficient fibroblasts stimulated 3D outgrowth and was associated with a significant increase in fibroblast proliferation, survival, motility and invasive capacity, as well as by activation of the *ras*-MAP kinase pathway (Laurent-Matha et al., 2005). Interestingly, all these stimulatory effects on fibroblasts were independent of cath-D proteolytic activity (Laurent-Matha et al., 2005).

The question remains, however, as to how cath-D induces such a major change in fibroblast phenotype. Since fibroblasts can become activated by either catalytically-active or -inactive cath-D or indeed by the secreted pro-cath-D precursor, its proteolytic activity is clearly not directly involved in the stimulatory effect (Laurent-Matha et al., 2005). At present, the only receptor known to interact with the secreted pro-cath-D is M6P/IGF2 receptor, which has a well defined function in the transport of various ligands *via* the endosomal pathway (Clague, 1998). However, the ability of this receptor to stimulate cellular responses *via* signaling pathways remains controversial although a recent study indicates that it may transduce IGF2 mitogenic activity *via* the MAP kinase pathway (McKinnon et al., 2001).

Another possibility is that secreted pro-cath-D binds to an as yet unidentified cell surface receptor coupled to the MAP kinase transduction pathway. Indeed it has been proposed that such a receptor exists at the cell surface of cancer and endothelial cells to mediate cath-D mitogenic activity (Fusek et al. 1994; Laurent-Matha et al., 1998; Berchem et al., 2002). We therefore propose that cath-D may favor tumor progression not only by affecting the epithelial compartment, but also by promoting fibroblast outgrowth *via* a paracrine loop (Liaudet-Coopman et al., 2006). Under these circumstances, cath-D over-expressed and hyper-secreted by cancer cells may be captured *in vivo* by stromal cells, may promote proliferation and survival, may stimulate motility and invasion of fibroblasts and consequently may enhance tumor-host homeostasis.

Cath-D hyper-secreted by breast cancer cells, is captured by fibroblasts.

In addition to the degradation of the extracellular matrices, an increased stromal growth has also been described as occurring in tumors (Shekhar et al., 2001). Tumor fibroblasts confer both morphogenic and mitogenic induction of epithelial cells and further enhancement of growth and progression requires active angiogenesis (Shekhar et al., 2001). Therefore the factors required for the normal behaviour of fibroblasts are crucial. If these factors are over-expressed by cancer cells and can be captured by fibroblasts, then they might optimize the behaviour of fibroblasts thereby enhancing tumor development and progression.

Pro-cath-D secreted by breast cancer cells can be captured by fibroblasts (Laurent-Matha et al., 1998; Heylen et al., 2002; Laurent-Matha et al., 2005). According to these reports and to our previous report indicating a stimulatory effect of cath-D on tumor angiogenesis (Berchem et al., 2002), cath-D over-expressed and hyper-secreted by breast cancer cells might be a mitogen that induces stromal proliferation in tumors. Moreover, stromal fibroblasts contribute extensively to the serine protease and MMP activities in tumors (Christensen et al., 1997; Uria et al., 1997; Basset et al., 1999). We were unable to detect any significant change in the mRNA expression of uPA, PAI1, MMP2, MMP9, MMP14, TIMP-1 and TIMP-2 in cath-D transfected fibroblasts (Laurent-Matha et al., 2005). However, cath-D may, by stimulating the growth of fibroblasts, indirectly increase the level of the other proteases implicated in the degradation of the extracellular matrix. Conversely, the ability of extracellular matrix components to mediate expression of proteases has not yet been fully examined. It has been shown that interaction of human breast fibroblasts with collagen I increased the secretion of pro-cath-B, but not that of pro-cath-D (Koblinski et al., 2002).

Conclusion.

Our study demonstrates that cath-D plays a crucial role for outgrowth of fibroblasts in three-dimensional matrices, affecting cell proliferation, motility and invasion. We thus propose that cath-D may favor breast tumor progression not only by affecting the epithelial compartment but also by strongly promoting fibroblast outgrowth *via* a paracrine loop (Liaudet-Coopman et al., 2006). Cath-D over-expressed and hyper-secreted by cancer cells might be captured *in vivo* by fibroblasts, stimulating their growth, motility and invasion, therefore increasing the production of essential factors by fibroblasts, consequently enhancing homeostasis of the tumor. Its action in cancer seems to implicate its extracellular interaction with a cell surface membrane receptor. This receptor has not yet been identified, but it is clearly of considerable potential interest to clarify the mechanisms involved and to consider their medical applications. If we viewed the cancer state as a product of its micro-environment, identification of the factors that participated in tumor-host homeostasis is crucial for the development of new stromal therapy.

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